Missouri Department of Natural Resources

Biological Assessment and Fine Sediment Study

Eaton Branch
St. Francois County, Missouri

2003-2004

Prepared for:

Missouri Department of Natural Resources
Water Protection and Soil Conservation Division
Water Protection Program
Water Pollution Control Branch

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1.0 Introduction

Eaton Branch, St. Francois County, is a small stream that is approximately three miles long in the Ozark/Meramec Ecological Drainage Unit (**EDU**, Figure 1). The stream originates near Wortham, Missouri and flows to its confluence with Big River approximately one-quarter of a mile north of Leadwood, Missouri. Most of the original Eaton Branch, south of Missouri State Highway 8 (MO 8) is covered with what appears to be mine tailings and flow is intermittent or subsurface. Flow from north of MO 8 to the confluence with Big River appears to be permanent or semi-permanent. This study was conducted downstream of MO 8.

Because of the small size of Eaton Branch, the stream is considered a “class C” stream, which means it may cease flow but maintain permanent pools in dry weather (MDNR 2000). Cub Creek acted as a similar size control and is considered a “class P” stream with permanent flow. The “Use Designations” (MDNR 2000) for Eaton Branch and Cub Creek are for “Livestock and Wildlife Watering (**LWW**) and the Protection of Warm Water Aquatic Life, Human Health-Fish Consumption (**AQL**)”. Both streams are considered “General Warm Water Fisheries” (**GWWF,** MDNR 2000).

1.1 Justification

Eaton Branch is a tributary of Big River that drains the historic Leadwood tailings ponds in what is known as the Old Lead Belt. The Leadwood mines and mill began operation in 1894 and continued with little interruption until 1965. The tailings ponds consist of approximately 528 acres of inactive lead mine tailings (Fluor Daniel Environmental Services 1995). Two tailings retaining structures capture drainage in the area. Whether they are effective is not known. Runoff from these tailings ponds may influence the aquatic communities on Eaton Branch and subsequently Big River. While Eaton Branch has not been placed on the 303(d) list, approximately 40 miles of Big River is listed for lead and non-volatile suspended solids (**NVSS**) and 53 miles are listed for lead, presumably from mine tailings runoff.

Abandoned mines may discreetly impair aquatic communities. Water runoff during rain events erodes mine wastes, which increase sedimentation in some lower portions of Big River. Tailings are generally fine sediments (ca. <2.0 mm) and have been found downstream in some portions of Big River. Fine sediments and silt clog the interstitial voids between the larger particles in the substrate and can have destructive effects on invertebrates and fish communities (Chutter 1969; Murphy et al. 1981; Berkman and Rabeni 1987; Smale et al. 1995).

Metals such as copper, iron, lead, and zinc have been detected in aquatic fauna in areas of Big River (Meneau 1997). Lead concentrations in fish tissues have resulted in fish consumption advisories. The metals composition (i.e. character) of the sediment may influence macroinvertebrate communities as well. Lead is toxic to all phyla of aquatic biota, but its’ toxic action differs by species and physiological state and by physical and chemical variables (Eisler 1988). Metals can affect aquatic organisms as toxic substances in water and sediment or as a toxicant in the food chain (Sorensen 1991; Rainbow 1996).

Clements (1991) found a lowered percent composition or elimination of Ephemeroptera and increased abundance of Chironomidae, especially Orthocladiinae, as well as Hydropsychidae (net-spinning caddisflies), downstream from metals impacts in the absence of organic pollution. Besser et al. (1987) stated that aquatic organisms in tributaries of Big River located downstream from tailings piles contained concentrations of lead, cadmium, and other heavy metals. Kramer (1976) and Jenett et al. (1981) reported elevated levels of lead and zinc in Flat River Creek, St. Francois County, a tributary to Big River. Concentrations of lead and zinc were elevated within algae, crayfish, and minnows from lower Flat River Creek. They believed the sources were brought to Flat River Creek via tributaries that drained Elvins and Federal tailings piles.

In 2001, the MDNR, ESP, Water Quality Monitoring Section (**WQMS**) identified Elvins Tailings Pile as a potential source of lead and zinc laden sediment found in Flat River Creek (MDNR 2001). Areas downstream from tailings piles on the lower portion of Big River were shown to be greatly impaired, potentially by fine sediment or heavy metals concentrations on the substrate (Zachritz 1978; Czarnezki 1987; Meneau 1997; MDNR 2003a). It is our intention to determine if Eaton Branch is impaired by past mining influences.

A biological assessment, stream habitat assessment, and fine sediment study plan (Appendix A) were developed for the Water Protection Program (**WPP**), Water Protection and Soil Conservation Division (**WPSCD**), Missouri Department of Natural Resources (**MDNR**). The study was coordinated and conducted by the Aquatic Bioassessment Unit of the WQMS, Environmental Services Program (**ESP**), MDNR. Kenneth B. Lister, David Michaelson, and the staff of the Water Quality Monitoring Section conducted the study. A biological assessment, stream habitat assessment, and fine sediment study were conducted on Eaton Branch and Cub Creek (control).

1.2 Purpose

To determine if Eaton Branch, St. Francois County is impaired by past mining influences.

1.3 Objectives

1) Assess the macroinvertebrate community integrity and water quality in Eaton Branch, St. Francois County.

2) Assess the stream habitat quality of Eaton Branch, St. Francois County.

3) Assess the relative quantity or actual character (metals content) of fine sediment sized particles at Eaton Branch, St. Francois County.

1.4 Tasks

1) Conduct a biological assessment for Eaton Branch, St. Francois County and Cub Creek, Washington County.

2) Conduct a stream habitat assessment on Eaton Branch and Cub Creek.

3) Conduct a fine sediment percentage estimation and characterization study on Eaton Branch and Cub Creek.

1.5 Null Hypotheses

Eaton Branch macroinvertebrate communities and biological criteria metrics will be similar to Cub Creek.

Water quality at Eaton Branch will be similar to Cub Creek and within acceptable Water Quality Standards (MDNR 2000).

Stream habitat assessment at Eaton Branch will be comparable to the stream habitat control station.

There will be no significant difference ($p > 0.05$) in the fine sediment percentage estimates or character between Eaton Branch and Cub Creek. The metals content will be below Probable Effects Levels (PELs; Ingersoll et al. 1996).

2.0 Methods

The study area, station descriptions, EDUs, and land use are identified. Study timing is outlined. Methods for stream habitat assessment are discussed. Biological assessment collection and analyses are introduced. Physicochemical water collection and analyses methods are defined.

2.1 Study Timing

Sampling was conducted for up to three seasons; spring 2003, fall 2003, and spring 2004. One reconnaissance (water only), two biological assessments, one stream habitat assessment, and one fine sediment study were conducted on Eaton Branch and Cub Creek.

A reconnaissance and physicochemical water sampling were conducted April 4, 2003. Biological assessments and the fine sediment study took place October 1 and 2, 2003 and March 23, 2004. Stream habitat assessments were conducted on Eaton Branch and Cub Creek in March 2004.

2.2 Station Locations and Descriptions

Two stations were used in this project (Figure 1, Table 1). The test station on Eaton Branch, St. Francois County was located downstream of Missouri State Highway 8, approximately 0.5 miles north of Leadwood, Missouri. Cub Creek, Washington County is a class P stream with permanent flow (MDNR 2000) and was used as the similar-size control stream. The station on Cub Creek was located approximately 1.5 miles north of Courtois, Missouri and 0.25 miles north of Washington County Road C.

Table 1
Location and Descriptive Information for Eaton Branch and Cub Creek Stations, 2003-2004

Stream-Station Number	Location-Section, Township, Range	Description	County
Eaton Branch #1	SE ¼ of sec. 33, T. 37 N., R. 04 W.	Test station-upstream from Big River confluence	St. Francois
Cub Creek #1	SE ¼ of sec 32, T. 36 N., R. 01 W.	Similar-size control-upstream 1.0 mile Courtois Creek confluence	Washington

2.2.1 Ecological Drainage Unit

Eaton Branch and Cub Creek are in the Ozark/Meramec Ecological Drainage Unit (EDU, Figure 1). Ecological Drainage Units are delineated drainage units that are expected to contain similar aquatic communities and stream habitat conditions. Comparisons between similar-size streams in the same EDU should then be appropriate.

2.2.2 Land Use

Land cover throughout the entire Ozark/Meramec EDU was compared to the land cover of each station by it's 14-digit Hydrological Unit (**HU**, Table 2). Percent land cover data were derived from Thematic Mapper (out **TM**) satellite data collected between 1991 and 1993 and interpreted by the Missouri Resource Assessment Partnership (**MoRAP**). The implication of this comparison is that land use within the study area does not interfere with interpretation of the findings, such as comparing streams near cropland and others near forestland.

Table 2
Percent Land Cover in the Eaton Branch, Cub Creek, and Ozark/Meramec EDU

Stations	HUC-14	Urban	Crops	Grassland	Forest
Eaton Branch #1	07140104010006	1.2	1.5	47.9	44.9
Cub Creek #1	07140102040002	0	0	7	92.6
Ozark/Meramec EDU	--	1.3	1.7	28.5	67.1

Land use (cover) in the Eaton Branch was comparable to the EDU and to Cub Creek on a smaller scale. Eaton Branch and the EDU were relatively similar in all categories (Table 2). Eaton Branch and Cub Creek on the scale of the HU were not similar in land-cover. Eaton Branch had far less forest (44.9%) than Cub Creek (92.6%). Land use near Eaton Branch was affected by its proximity to Leadwood, Missouri and pasture. However, the riparian corridor was well forested surrounding Eaton Branch. On a smaller scale, the comparisons between Eaton Branch and Cub Creek should not be influenced by land use in the surrounding areas.

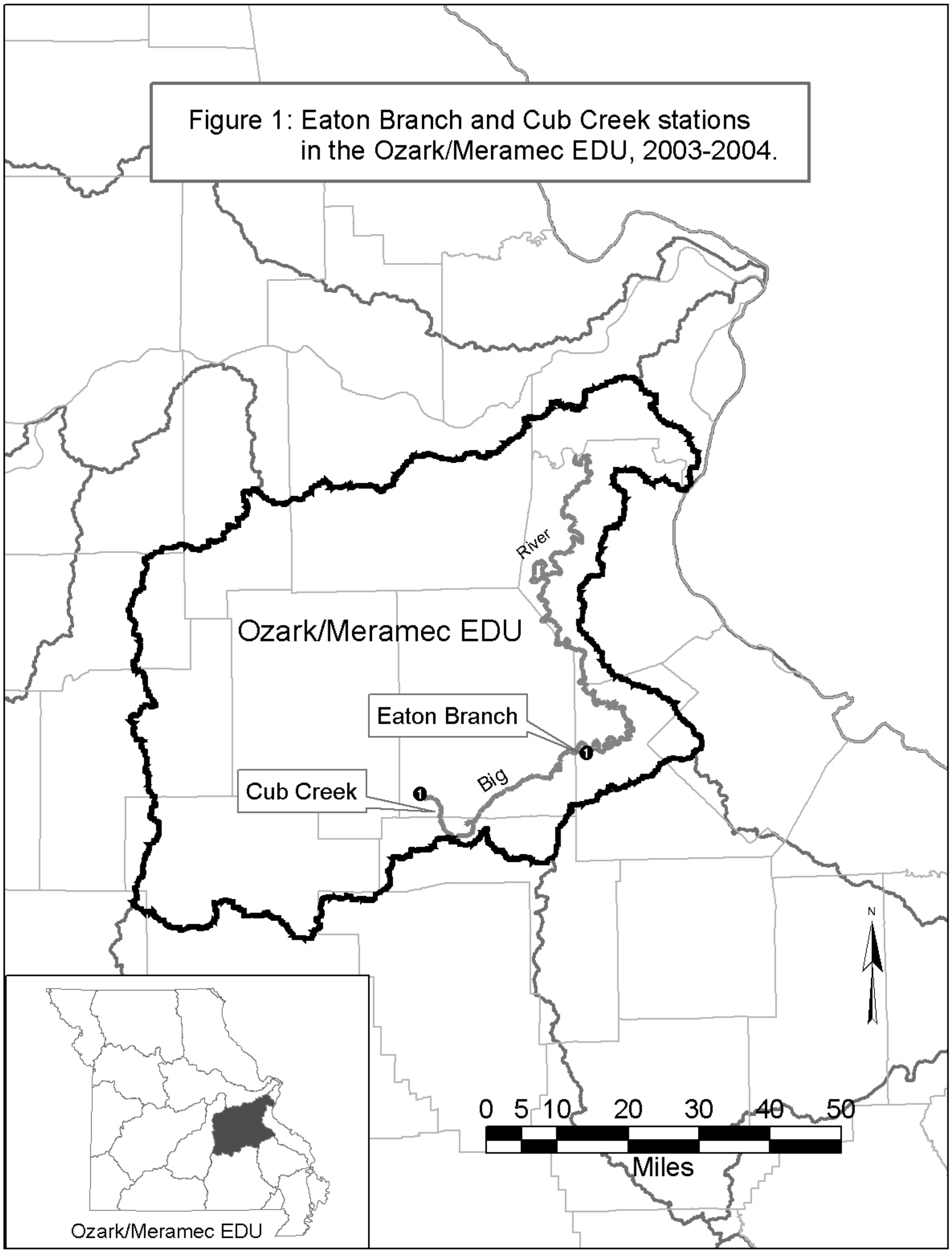


Figure 1: Eaton Branch and Cub Creek stations
in the Ozark/Meramec EDU, 2003-2004.
Ozark/Meramec EDU
River
Eaton Branch
Big
Cub Creek
N
0 5 10 20 30 40 50
Miles
Ozark/Meramec EDU

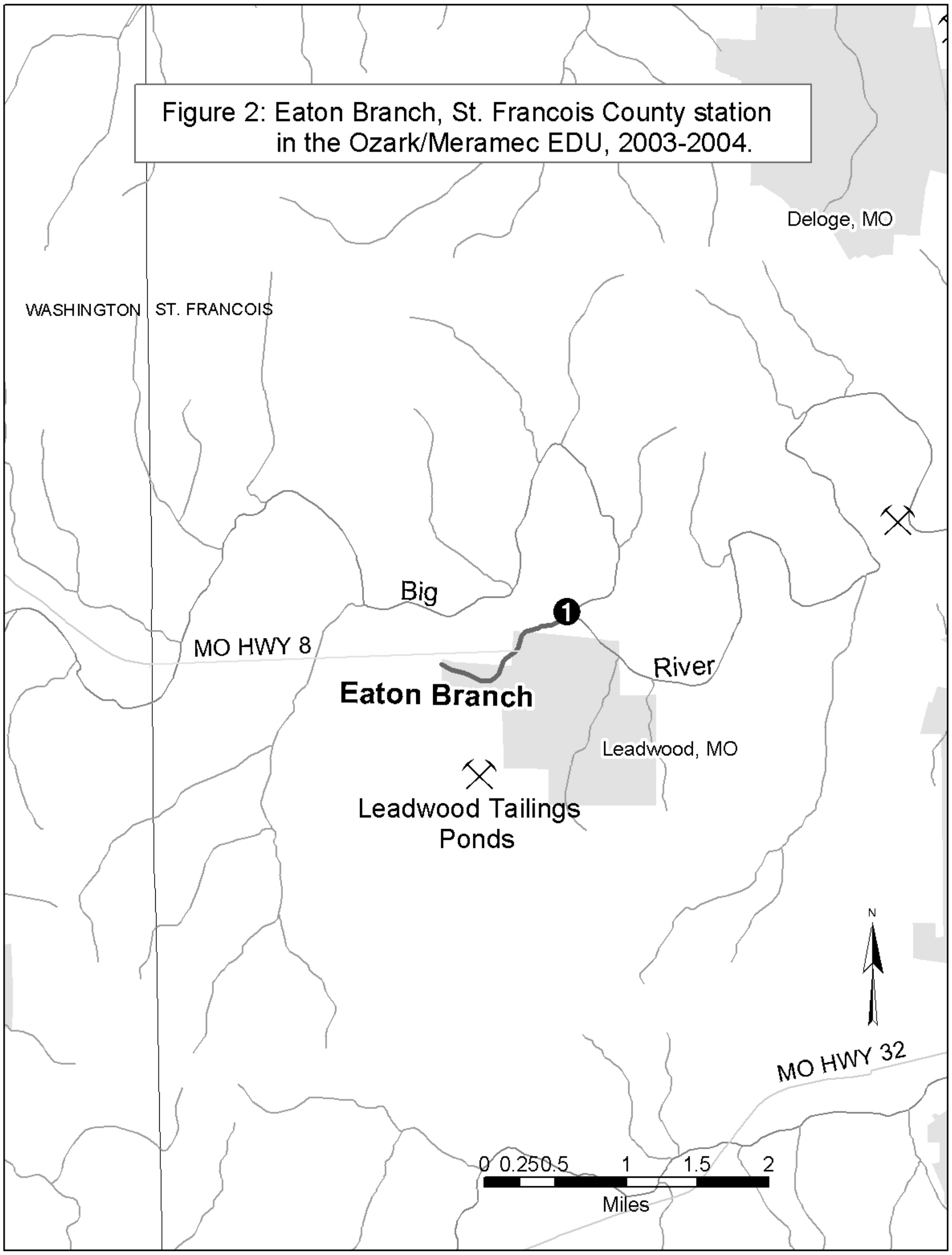


Figure 2: Eaton Branch, St. Francois County station
in the Ozark/Meramec EDU, 2003-2004.
Deloge, MO
WASHINGTON
ST. FRANCOIS
Big
River
MO HWY 8
Eaton Branch
Leadwood, MO
Leadwood Tailings
Ponds
N
MO HWY 32
0 0.250.5 1 1.5 2
Miles

2.3 Stream Habitat Assessment

The standardized Stream Habitat Assessment Project Procedure, MNDR-FSS-032 (**SHAPP**) was conducted on Eaton Branch and Cub Creek, as described for "Riffle/Pool Habitat" (MDNR 2003f). Components of the stream habitat were evaluated and total SHAPP scores were derived for each station. Scores were compared at Eaton Branch and Cub Creek (stream habitat control). If the SHAPP score at the test station was $\geq$75% of the stream habitat control, the test station was considered comparable to the quality of the stream habitat control and supporting the aquatic community.

2.4 Biological Assessment

Biological assessments were conducted for one station on Eaton Branch and one station on Cub Creek. Biological assessments consisted of macroinvertebrate community and physicochemical water sampling and analyses.

2.4.1 Macroinvertebrate Sampling and Analyses

Macroinvertebrates were sampled as described in the MDNR's Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure (**SMSBPP,** MDNR 2003e). Eaton Branch and Cub Creek are considered riffle/pool dominant streams and multiple habitats were sampled accordingly (MDNR 2003e). These included flowing water with coarse substrate (**CS**), non-flowing water with depositional substrates (**NF**), and rootmat (**RM**) habitats.

Macroinvertebrate community data are analyzed using three strategies. Stream Condition Index (**SCI**) scores, individual biological criteria metrics, and dominant macroinvertebrate families (**DMF**) were examined and compared between stations. All results were grouped by season and station.

The first comparison was of SCI scores by station and season. An SCI score was identified as a qualitative rank measurement of a stream's aquatic biological integrity (Rabeni et al. 1997). The SCI was further refined for reference streams within each EDU according to Biological Criteria for Perennial/Wadeable Streams (**BIOREF**, MDNR 2002b). Four primary biological criteria metrics were used to calculate the SCIs per station: 1) Taxa Richness (**TR**); 2) Ephemeroptera/Plecoptera/Trichoptera Taxa (**EPTT**); 3) Biotic Index (**BI**); and 4) Shannon Diversity Index (**SDI**). Metric (TR, EPTT, BI, SDI) scores were compared to the BIOREF scoring range (SCI Scoring Table, Tables 4 and 5) and rank scores (5, 3, 1) were assigned to each metric. Rank scores were compiled for each station and SCIs were completed. For example, an SCI of 20-16 = fully supporting of the biological community; 14-10 = partially supporting of the biological community; and 8-4 = non-supporting of the biological community.

Secondly, individual biological criteria metrics (TR, EPTT, BI, SDI) values were examined compared to the BIOREF scoring range (Scoring Table, Tables 4 and 5) to identify unusual responses or interesting trends at each station. Variations in certain metrics may help illustrate impairment, if it exists, and potentially identify a source.

Thirdly, the dominant macroinvertebrate families (DMFs) as a percentage of the total number of individuals were evaluated. Eight DMFs were listed for each station by season. Dominance by certain families may illustrate impairment, if it exists, and potentially identify a source of impairment.

A complete taxa list (Appendix B) was also included for each station and grouped by season. Presence or absence of individual taxa may be discussed based on their tolerance to impairment.

2.4.2 Physicochemical Water Sampling and Analyses

Physicochemical water samples were handled according to appropriate MDNR, ESP Standard Operating Procedures (**SOPs**) and Project Procedures (**PPs**) for sampling and analyzing physicochemical water samples. Results are reported for physicochemical water variables by season and station.

Spring 2003, fall 2003, and spring 2004 physicochemical water parameters measured in the field were temperature (C^0), pH, conductivity (uS/cm), dissolved oxygen (mg/L), and discharge (cfs).

Water was sampled according to the SOP MDNR-FSS-001 Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations (MDNR 2003d). Samples were collected and kept on ice for transport to the ESP. Water samples were collected and returned to the ESP laboratory for turbidity, hardness ($CaCO_3$), ammonia-nitrogen, nitrate+nitrite-nitrogen, Total Kjeldahl Nitrogen (**TKN**), sulfate (spring 2003 only), chloride, total phosphorus, dissolved barium (spring 2003 and fall 2003 only), dissolved cadmium, calcium, dissolved copper, dissolved iron, dissolved lead, dissolved magnesium, dissolved nickel, and dissolved zinc. The WQMS Biology Laboratory analyzed turbidity. All remaining samples were delivered to the ESP Chemical Analysis Section (**CAS**) in Jefferson City, Missouri for analyses.

Physicochemical data were compared between Eaton Branch and Cub Creek. Results were also compared with acceptable limits according to Missouri's Water Quality Standards (**WQSs**, MDNR 2000).

Interpretation of acceptable limits within the WQSs may be dependent on a stream's classification and its beneficial use designations (MDNR 2000). Eaton Branch is a class C stream downstream from MO 8 and Cub Creek is a class P stream. Both have designated uses for LWW and AQL. They are also considered General Warm Water Fisheries (GWWF). Furthermore, acceptable limits for some variables may be dependent on the rate of exposure. These exposure or toxicity limits are based on the lethality of a toxicant given long (chronic toxicity, **c**) or short-term exposure (acute toxicity, **a**). Water hardness (mg/L $CaCO_3$) concentrations were necessary to further determine acceptable limits, based on the solubility of heavy metals.

2.4.3 Discharge

Stream flow was measured using a Marsh-McBirney Flowmate™ flowmeter at each station. Velocity and depth measurements were recorded at each station according to SOP, MDNR-WQMS-113 Flow Measurement in Open Channels (MDNR 2003c).

2.5 Fine Sediment

In-stream deposits of fine sediment (i.e. particle size ca. <2 mm) were estimated for percent coverage per area and characterized for composition of total recoverable metals (**TR**, ug/kg). The ESP, CAS conducted metals analyses.

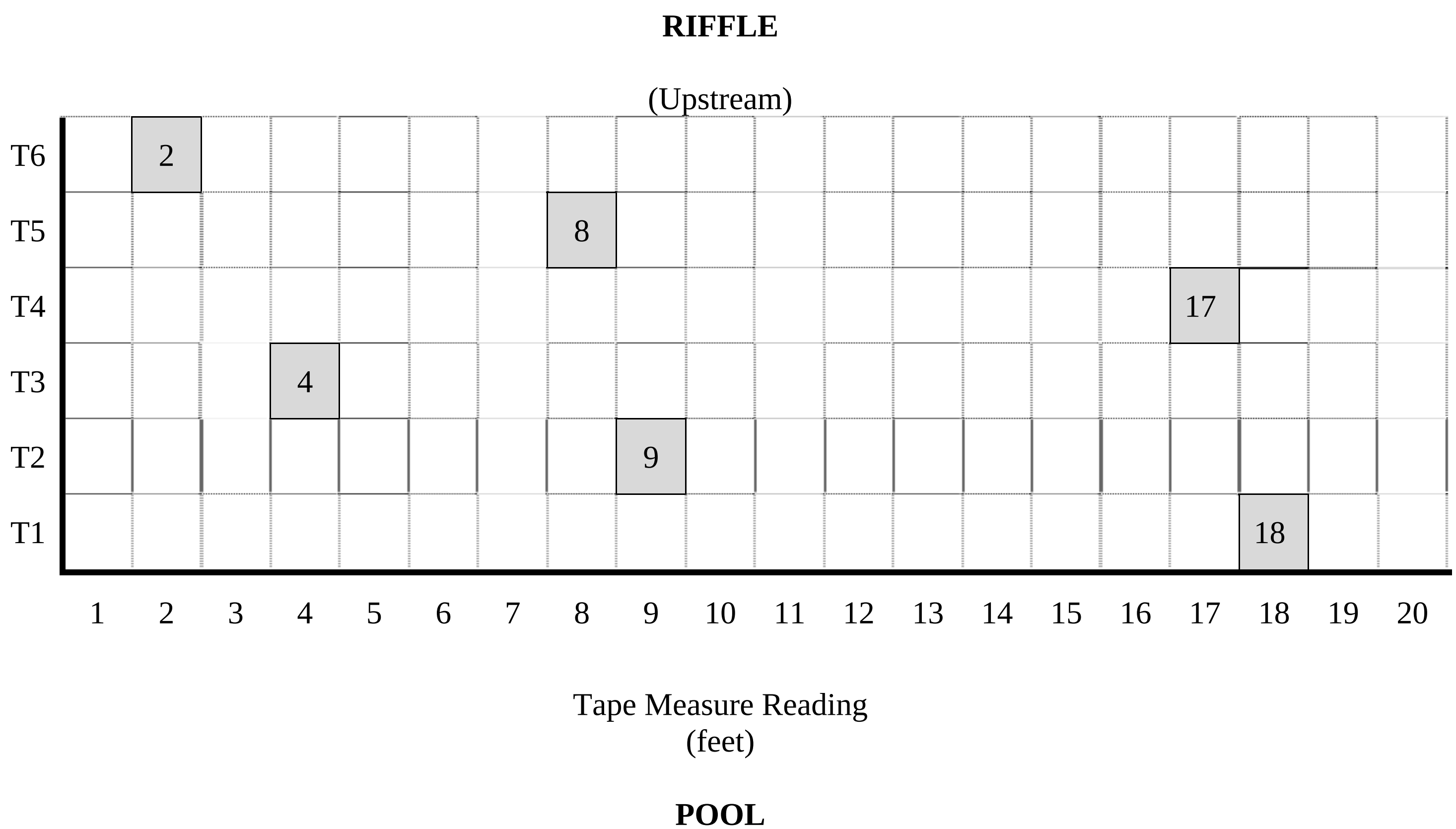
2.5.1 Fine Sediment Estimations

The relative percentage of fine sediment (<2.0 mm) was visually estimated for each station. The visual estimates were conducted within a metal square (**quadrat**) that was randomly located in sample areas called grids (Figure 3). Each station contained three grids. This method allowed for estimation and comparison of benthic fine sediment between stations.

In order to ensure sampling method uniformity, grids were located at lower margins of riffles or runs and the upper margin of pool habitats in areas of relatively laminar flow. This arrangement or placement of grids was similar to previous fine sediment assessment projects done by the WQMS (MDNR-WQMS Reports: Flat River 2001; Bull Creek 2002a; Upper Big River 2001-2002). Water velocity was no greater than 0.5 feet per second (**fps**), which allows fine sediment sized particles (<2.0mm) to settle according to the Hjulstrom Diagram (1939) for threshold transport and settling velocities. A Marsh McBirney flow meter was used to determine the velocity. Depths did not exceed three (3.0) feet. Grids did not include eddies, bends, downstream of vegetation, or large obstructions that have turbulent flow.

Once a suitable area was identified, a virtual grid was constructed (Figure 3). A 100’ tape measure anchored across the stream symbolized the downstream edge of a virtual grid of six contiguous transects. Each transect was 12” deep and as wide as the useable grid and was identified by holding a retractable tape measure perpendicular to the 100’ tape. The useable grid width included the width of the stream with relatively laminar flow that excluded eddies, vegetation, and large obstructions. Random numbers, equating to one foot increments, were drawn to determine where the quadrat was placed along each transect. The quadrat was placed within the transect, with the downstream edge contacting the downstream transect edge. Two observers estimated/recorded the percent of fine sediment within the quadrat. The estimates were accepted and recorded if the two observations were within a ten percent margin of error or rejected and repeated until the margin of error was reached. Another random number was drawn and the quadrat was randomly placed in the next transect upstream where the next observation was made. This continued until fine sediment was estimated in each of the six quadrats (one per transect).

Figure 3: Virtual grid of transects (T) and quadrats (in gray, numbered) for estimating percent fine sediment. Example: stream 20’ wide; quadrat placement based on random numbers (e.g. 18, 9, 4, 17, 8, 2).



2.5.2 Fine Sediment Character

Fine sediment was characterized by identifying its content of total recoverable cadmium, lead, and zinc (ug/kg). One composite sample of the fine sediment was collected at each grid (downstream from riffle). Each composite consisted of three (3) two-ounce grab samples of fine sediment sized particles that were dredged from the substrate and placed into an eight-ounce glass jar. Dredging depth did not exceed two inches. The flat surface of the two-ounce jar lid was used to retain the fine sediment while the jar was retrieved through the water column. If fine sediment was not found in sufficient quantities within the grid, a representative composite collection was collected near the study grid. A total of three composite samples were collected and analyzed for each station. Samples were kept on ice and delivered to the ESP, CAS in Jefferson City, Missouri to be analyzed.

2.5.3 Fine Sediment Data Analyses

The fine sediment estimates and characterizations were compared between control stations and test stations and acceptable levels.

The mean of the two fine sediment estimations per quadrat was entered for testing fine sediment. Statistical analyses were conducted on the differences between stations using SigmaStat, Version 2.0 (1997). The Mann-Whitney Rank Sum test was conducted on the fine sediment estimations per station. This illustrated significant differences ($p<0.05$) between sample stations. Data for all quadrats in each station ($n=18$ quadrats) were included in the comparison between stations. Thirty-six observations of fine sediment were used in the fine sediment estimation comparison for Eaton Branch and Cub Creek.

The character of the fine sediment was compared using two methods. The first was a comparison of the amounts of heavy metals in the sediment between Eaton Branch and Cub Creek. The Student t-test was used to identify differences ($p<0.05$) in the mean concentrations of sediment metals per station ($n=3$). Second, the levels of heavy metals found at all stations were compared to Probable Effects Levels (**PELs**, Ingersoll et al. 1996). Data from three composite samples were used in the analyses for each station.

2.6 Quality Control

Quality control was conducted according to MDNR Standard Operating Procedures (**SOP**s) and Project Procedures (**PPs**). Duplicate biological assessments and macroinvertebrate and physicochemical water sampling and analyses were conducted on Cub Creek for quality control during the spring 2004 season.

3.0 Results and Analyses

Results include stream habitat assessments, biological assessments, discharge, and fine sediment estimation and characterizations for Eaton Branch and Cub Creek.

3.1 Stream Habitat Assessment

The stream habitat was assessed at Eaton Branch and Cub Creek (Table 3). Eaton Branch had a score of 120, while Cub Creek scored 142. Eaton Branch stream habitat was 84.5 percent the quality of the Cub Creek stream habitat control.

Table 3
Stream Habitat Assessment Scores for Cub Creek and Eaton Branch, Spring 2004
(March 23 and 24, 2004)

Stations	Eaton Branch #1	Cub Creek #1
Stream Habitat Score	120	142
Percent of Control (Cub Creek)	84.5	100

3.2 Biological Assessment

Biological assessments consisted of macroinvertebrate community analyses and physicochemical water quality to determine the ecological integrity of each station.

3.2.1 Macroinvertebrate Community Analyses

Macroinvertebrate community data were analyzed using three strategies. Stream Condition Index (**SCI**) scores, individual biological criteria metrics, and dominant macroinvertebrate families (**DMF**) were examined and compared. All results were grouped by season and station.

The SCI score at Eaton Branch (12) was not similar to the SCI at Cub Creek (20) in the fall (Table 4). This score indicated that Eaton Branch is partially supporting of the biological community, while Cub Creek is fully supporting of the biological community.

Individual metrics were not similar between Eaton Branch and Cub Creek in the fall, with one exception (Table 4). Taxa richness at Eaton Branch (39) was less than half of Cub Creek (98). The EPT taxa at Eaton Branch (7) were nearly four times less than Cub Creek (27). The BI was similar between Eaton Branch (5.33) and Cub Creek (4.43). The SDI at Eaton Branch (2.08) was much less than Cub Creek (3.41) in the fall.

Table 4
Fall 2003 Biological Criteria (n=7) Metric Scores and Stream Condition Index (SCI) Scores for Eaton Branch and Cub Creek Stations. (SCI Scoring Table in light gray)

Station	Eaton Branch #1	Cub Creek #1	Score 5	Score 3	Score 1
	Sample # 0318730	Sample # 0318731			
Taxa Richness	39 (Score =3)	98 (Score = 5)	>78	78-39	<39
EPT Taxa	7 (Score = 1)	27 (Score = 5)	>21	21-10	<10
Biotic Index	5.33 (Score = 5)	4.43 (Score = 5)	<5.78	5.78-7.89	>7.89
Shannon DI	2.08 (Score = 3)	3.41 (Score = 5)	>3.08	3.08-1.54	<1.54
SCI	12	20	20-16	14 - 10	8-4
Biological Support Category	Partial	Full	Full	Partial	Non

SCI scores were not similar between Eaton Branch (12) and the Cub Creek duplicates 1a (20) and 1b (16) in the spring (Table 5). Eaton Branch was shown to be partially supporting of the biological community. Cub Creek 1a and 1b duplicates were similar to each other and both were fully supporting of the biological community.

Individual metrics were not similar between Eaton Branch and the Cub Creek duplicates in the spring, with one exception (Table 5). Taxa Richness at Eaton Branch (48) was approximately half of the Cub Creek duplicates (97, 88). The EPT taxa at Eaton Branch (9) were approximately one-third of the Cub Creek duplicates (30, 27). The BI at Eaton Branch (4.53) was similar to the duplicates (4.13, 4.75). The SDI at Eaton Branch (1.67) was approximately one-third of the Cub Creek duplicates (3.33, 3.47). The duplicates at Cub Creek were consistent and similar.

Table 5
Spring 2004 Biological Criteria (n=6) Metric Scores and Stream Condition Index (SCI) Scores for Eaton Branch and Cub Creek Stations. (SCI Scoring Table in light gray)

	Eaton Branch #1	Cub Creek #1a	Cub Creek #1b	Score 5	Score 3	Score 1
	Sample # 0418659	Sample # 0418660	Sample # 0418661			
Taxa Richness	48 (Score = 3)	97 (Score = 5)	88 (Score = 3)	>92	92-46	<46
EPT Taxa	9 (Score = 1)	30 (Score = 5)	27 (Score = 3)	>29	29-14	<14
Biotic Index	4.53 (Score = 5)	4.13 (Score = 5)	4.75 (Score = 5)	<5.80	5.80-7.90	>7.90
Shannon DI	1.67 (Score = 3)	3.33 (Score = 5)	3.47 (Score = 5)	>3.32	3.32-1.66	<1.66
SCI	12	20	16	20-16	14 - 10	8-4
Biological Support Category	Partial	Full	Full	Full	Partial	Non

Eaton Branch and Cub Creek shared one dominant family (Chironomidae) in the fall (Table 6). Psephenid and elmid beetles; heptageniid, baetid, isonychiid, and caenid mayflies were found in Cub Creek, however, were not among the dominant taxa in Eaton Branch.

Eaton Branch and the Cub Creek duplicates shared only four dominant families in the spring (Table 7). Eaton Branch was dominated by Chironomidae (75.3), as were Cub Creek 1a (39.3) and 1b (47.9) duplicates, however, at much lower percentages. Tubificids were among the dominant taxa at Eaton Branch (5.2) and Cub Creek 1b (2.1). Nemourid stoneflies were shared in dominance at Eaton Branch (5.1) and the Cub Creek duplicates 1a (8.5) and 1b (3.4). Empidid dipterans were found at Eaton Branch (1.2) and Cub Creek 1a (2.0) and 1b (2.1). Interestingly, elmid beetles, leuctrid stoneflies, ephemerellid and heptageniid mayflies were among the dominant families at Cub Creek, but were not among the dominant families in Eaton Branch. Duplicates were similar suggesting a lack of bias in sampling.

Table 6
Dominant Macroinvertebrate Families (DMF) as a Percentage of the Total Number of Individuals per Station, Fall 2003

Station	Eaton Branch #1	Cub Creek #1
Sample Number	0318730	0318731
Chironomidae	53.6	13.4
Leptoceridae	14.9	--
Tubificidae	9.3	--
Coenagrionidae	8.6	--
Asellidae	2.8	--
Tipulidae	2.6	--
Hydroptilidae	1.5	--
Calopterygidae	1.0	--
Psephenidae	--	24.5
Elmidae	--	20.7
Heptageniidae	--	8.0
Baetidae	--	3.8
Isonychiidae	--	3.8
Hyalellidae	--	2.4
Caenidae	--	2.1

Table 7
Dominant Macroinvertebrate Families (DMF) as a Percentage of the Total Number of Individuals per Station, Spring 2004

Station	Eaton Branch #1	Cub Creek #1a	Cub Creek #1b
Sample Number	0418659	0418660	0418661
Chironomidae	75.3	39.3	47.9
Tubificidae	5.2	--	2.1
Nemouridae	5.1	8.5	3.4
Simuliidae	2.7	--	--
Coenagrionidae	1.8	--	--
Ceratopogonidae	1.2	--	--
Caenidae	1.2	--	--
Empididae	1.2	2.0	2.1
Elmidae	--	14.5	13.5
Leuctridae	--	13.8	7.0
Ephemerellidae	--	2.6	2.0
Arachnoidea	--	1.9	--
Heptageniidae	--	1.9	3.0

3.2.2 Physicochemical Water Variables

Physicochemical water variables for Eaton Branch and Cub Creek were compared to each other and to WQSs (Table 8). There were notable results in sulfate + chloride, dissolved

cadmium, and dissolved zinc levels between Eaton Branch and Cub Creek. Sulfate and chloride were detected above WQSs (MDNR 2000) in the Eaton Branch water sample during the spring 2003 reconnaissance (Table 8). Sulfate was 219 mg/L and chloride reached 12.7 mg/L for a combined total of 231.7 mg/L. The chronic toxicity (**c**) limit is 230 mg/L for sulfate + chloride in the WQSs (MDNR 2000). Water from Cub Creek was not analyzed for sulfate. Chloride was much lower at Cub Creek than Eaton Branch.

Table 8
Physicochemical Water Variables for Eaton Branch and Cub Creek by Season: Spring 2003, Fall 2003, and Spring 2004. (Units mg/L unless otherwise noted; **Bold** above WQS; **c** = chronic, **a** = acute toxicity (MDNR 2000; or trend)

Station Variable-Date	Eaton Branch, Spring 2003 Reconn. 4-2-2003	Eaton Branch #1, Fall 2003 Test 10-1-2003	Cub Creek, #1, Fall 2003 Control 10-2-2003	Eaton Branch #1, Spring 2004 Test 3-23-2004	Cub Creek, #1a/1b, Spring 2004 Control 3-23-2004
Phys/Chem Sample No.	0300559	0300570	0300574	0411022	0411023/ 0411024
pH (Units)	7.9	8.0	8.0	7.9	8.4
Temperature (C^0)	13.8	12.0	15.0	8.5	8.5
Conductivity (uS)	758	984	414	755	324
Dissolved Oxygen	10.2	9.33	9.36	10.9	11.4
Discharge (cfs)	3.00	0.68	2.43	1.50	12.1
Turbidity (NTUs)	3.36	1.73	<1.00	2.39	<1.00/<1.00
Hardness	426	597	234	422	185/ 182
Nitrate/Nitrite-N	<0.05	0.03	0.08	0.03	0.05/ 0.05
TKN	0.40	0.21	0.06	0.22	<0.05/<0.05
Ammonia-N	<0.05	<0.03	<0.03	<0.03	<0.03/<0.03
Sulfate	**219 (c)**	--	--	--	--
Chloride	**12.7 (c)**	11.3	2.70	11.8	2.28/ 2.38
Total Phosphorus	<0.05	<0.01	<0.01	<0.01	<0.01/<0.01
Barium (ug/L)-Dis.	45.7	40.1	70.5	--	--
Cadmium (ug/L)-Dis.	**5.00**	**4.78**	<0.25	**5.07**	<0.25/<0.25
Calcium-Dis.	98.9	148	47.0	102	38.2/ 37.9
Copper (ug/L)-Dis.	<10	<5.00	<5.00	2.14	0.88/ 0.55
Iron (ug/L)-Dis.	<10	5.55	1.81	--	--
Lead (ug/L)-Dis.	<2	5.03	0.51	3.83	<0.25/<0.25
Magnesium-Dis.	43.4	55.3	28.3	40.6	21.7/ 21.3
Nickel (ug/L)-Dis.	--	--	--	15.1	0.91/ 0.82
Zinc (ug/L)-Dis.	**1800 (c, a)**	**1720 (c, a)**	<1.00	**1240 (c, a)**	2.75/ 1.52

Dissolved cadmium was present at Eaton Branch, but not at Cub Creek (Table 8). In the spring of 2003, dissolved cadmium reached 5.0 ug/L. The fall sample contained 4.78 ug/L and the spring 2004 sample contained 5.07 ug/L. The chronic toxicity level of dissolved cadmium is 15.5 ug/L for General Warm-Water Fisheries (GWWF) with a hardness >200 mg/L Ca CO_3. The WQS (MDNR 2000) was not exceeded at Eaton Branch. Dissolved cadmium was not detected (<0.25 ug/L) in Cub Creek.

Dissolved zinc in the water was clearly different between Eaton Branch and Cub Creek (Table 8). Eaton Branch exceeded WQSs (MDNR 2000) on all three visits. Dissolved zinc was 1800 ug/L in spring 2003, 1720 ug/L in fall 2003, and 1240 ug/L in spring 2004. Chronic (433 ug/L) and acute (479 ug/L) Water Quality Standards (MDNR 2000) for a GWWF at a hardness >200 mg/L $CaCO_3$ were greatly exceeded at Eaton Branch. Cub Creek had a dissolved zinc concentration of <1.0 ug/L during the fall and 2.75 ug/L and 1.52 ug/L in the spring duplicates.

3.2.2.1 Discharge

Discharge was relatively similar between Eaton Branch and Cub Creek (Table 8). The spring 2003 discharge was 3 cfs at Eaton Branch. Eaton Branch discharge in fall 2003 was 0.68 cfs, while Cub Creek discharge was 2.43 cfs. Discharge at Eaton Branch in the spring was 1.50 cfs, while Cub Creek discharge was 12.1 cfs.

3.3 Fine Sediment

The fine sediment study consisted of comparisons between Eaton Branch and Cub Creek in the amount of fine sediment and sediment character (metals concentration). Statistical tests were conducted on either means or medians, however, tables contain mean values.

3.3.1 Fine Sediment Estimations

Fine sediment estimations were much higher at Eaton Branch than at Cub Creek (Table 9). The mean of fine sediment estimated was 78 (s.d.$\pm$21) percent at Eaton Branch and four (4; s.d.$\pm$3) percent at Cub Creek. The difference between the means is 74 percent. A SigmaStat report sheet for fine sediment statistical test is included as Appendix C. The normality test failed ($p<0.001$) using the student t-test, so a Mann-Whitney rank sum test was conducted between the medians at the two streams. Eaton Branch had significantly more ($p<0.001$; $T = 495.000$) fine sediment on the substrate than did Cub Creek.

3.3.2 Fine Sediment Character

The character (heavy metals concentrations) of the sediment was much different at Eaton Branch than at Cub Creek. High concentrations of cadmium, lead, and zinc were found in the fine sediment substrate at Eaton Branch. Cub Creek fine sediment metals concentrations were low. Concentrations of heavy metals in the sediment were compared to Probable Effects Levels (PELs, Ingersoll et al. 1996).

Table 9
Fine Sediment Estimates (Percent) for Stations/Grid and Quadrat;
Mean and Standard Deviation (s.d.) per Station.
(Six quadrats per grid or 18 per station)

Grid-Quadrat	Eaton Branch #1 Test Station October 1, 2003 (%)	Cub Creek #1 Control Station October 2, 2003 (%)
1-1	66	5
1-2	90	6
1-3	96	1
1-4	96	2
1-5	90	2
1-6	82	2
2-1	38	4
2-2	96	6
2-3	96	1
2-4	44	10
2-5	80	2
2-6	96	8
3-1	28	1
3-2	88	10
3-3	83	10
3-4	91	2
3-5	68	6
3-6	86	1
Mean Percentage	**78**	**4**
s.d.	21	3

The mean concentration of cadmium in the sediment was much higher at Eaton Branch than at Cub Creek (Table 10). The mean amount of cadmium in the sediment at Eaton Branch was 67,100 ($\pm$10,802) ug/kg while Cub Creek samples contained 60 ($\pm$5.3) ug/kg. The difference between means was 67,040 ug/kg. A t-test was conducted on the cadmium concentrations in the sediment (Appendix C). There was a significant difference ($p=0.001$; $t = 10.750$, d.f. = 4) in the concentrations of cadmium in the fine sediment sized particles at Eaton Branch and Cub Creek. The PEL for sediment cadmium (3,200 ug/kg) was greatly exceeded at Eaton Branch.

Table 10
Cadmium Levels (ug/kg) in Sediment per Grid; Means and Standard Deviations (s.d.) per Station. Sediment Cadmium Probable Effects Level (PEL)=3,200 ug/kg (Ingersoll et al. 1996). **Bold**=above PEL

	Eaton Branch #1	Cub Creek #1
Grid 1	78,900	55
Grid 2	57,700	61
Grid 3	64,700	66
Mean	**67,100**	60
s.d.	10,802	5

The mean concentration of lead in the sediment was much higher at Eaton Branch than at Cub Creek (Table 11). The mean amount of lead in the sediment at Eaton Branch was 2,243,333 (993,244) ug/kg, while Cub Creek samples contained 12, 843 ($\pm$9,844) ug/kg. The difference between means was 2,230,490 ug/kg. A t-test was conducted on the lead concentrations in the sediment (Appendix C). There was a significant difference ($p=0.018$; $t = 3.889$ with 4 d.f.) in the concentration of lead in the fine sediment sized particles at Eaton Branch and Cub Creek. The PEL for sediment lead (82,000 ug/kg) was greatly exceeded at Eaton Branch.

Table 11
Lead Levels (ug/kg) in Sediment per Grid; Means and Standard Deviations (s.d.) per Station. Sediment Lead Probable Effects Level (PEL)=82,000 ug/kg (Ingersoll et al. 1996). **Bold**=above PEL

	Eaton Branch #1	Cub Creek #1
Grid 1	2,490,000	24,200
Grid 2	1,150,000	7,560
Grid 3	3,090,000	6,770
Mean	**2,243,333**	12,843
s.d.	993,244	9,844

The mean concentration of zinc in the sediment was higher at Eaton Branch than Cub Creek (Table 12). The mean amount of zinc in the sediment at Eaton Branch was 2,013,333 ($\pm$579, 511) ug/kg, while Cub Creek samples contained 16, 833 ($\pm$929) ug/kg. The difference between means was 1,996,500 ug/kg. A t-test was conducted on the zinc concentrations in the sediment (Appendix C). There was a significant difference ($p=0.004$; $t = 5.967$ with 4 d.f.) in the concentration of zinc in the fine sediment sized particles at Eaton Branch and Cub Creek. The PEL for sediment zinc (540,000 ug/kg) was greatly exceeded at Eaton Branch.

Table 12
Zinc Levels (ug/kg) in Sediment per Grid; Means and Standard Deviations (s.d.) per Station. Sediment Zinc Probable Effects Level (PEL)=540,000 ug/kg (Ingersoll et al. 1996). **Bold**=above PEL

	Eaton Branch #1	Cub Creek #1
Grid 1	2,680,000	17,900
Grid 2	1,730,000	16,200
Grid 3	1,630,000	16,400
Mean	**2,013,333**	16,833
s.d.	579,511	929

4.0 Discussion

The discussion includes the stream habitat assessment, biological criteria metrics, physicochemical water variables, and fine sediment quantity and character study. The macroinvertebrate response may illustrate an association with several variables.

4.1 Stream Habitat Assessment

Stream habitat at Eaton Branch was considered to be comparable to the stream habitat control. It appears that the stream habitat in general did not obviously impair the biological community.

4.2 Biological Criteria Metrics

Eaton Branch individual rank scores were much lower than Cub Creek scores during both seasons. This indicated that a substantial and constant influence was affecting the community. Total SCI scores at Eaton Branch and individual metric SCI scores were identical (Table 4 and Table 5) during both seasons. This suggested that the influence on Eaton Branch was consistent between seasons. Results indicated that Eaton Branch was partially supporting of the biological community during both seasons. The macroinvertebrate community at Eaton Branch was not similar to Cub Creek.

By interpreting the biological criteria metrics, it appeared that the macroinvertebrate community at Eaton Branch had fewer EPT taxa, was less diverse, and less evenly distributed than Cub Creek and the BIOREF streams. However, the BI at Eaton Branch was similar and consistent with the control BI and scored well with the BIOREFs in both seasons. This suggested that organic pollution was probably not contributing to the impairment, short or long-term.

4.3 Physicochemical Water Quality

Sulfate and chloride levels combined were high at Eaton Branch and exceeded WQSs (MDNR 2000) during one season. Chronic toxicity levels (230 mg/L) were exceeded in the spring 2003 reconnaissance sample (231.7 mg/L). Since sulfate was sampled only during one season (spring 2003) it is not known if it is consistently high. However, chloride levels were consistent during all three seasons, which suggested that the input was continuous. High sulfate and chloride in the water are possible in intensively mined

areas. At such high levels, they may be affecting the ecological integrity. Dissolved cadmium was present during all three seasons at Eaton Branch, however, did not exceed WQSs (MDNR 2000). Dissolved cadmium is an expected component in an area such as the Big River watershed, where lead mining was prevalent for many years. Eaton Branch drains the Leadwood Tailings Piles (Ponds) which may explain the cadmium in the water column.

Dissolved zinc occurred at very high levels at Eaton Branch and greatly exceeded WQSs (MDNR 2000) during all three seasons. Dissolved zinc ranged from 1800 ug/kg in the spring of 2003 down to 1240 ug/kg in the spring of 2004. The chronic toxicity level (433 ug/L) and acute toxicity level (479 ug/L) were greatly exceeded for GWWF with a hardness >200 mg/L. Schmitt and Finger (1982) suggested that zinc was probably transported as a liquid, especially during high flow. It is possible that the dissolved zinc was eluting from the fine sediment on the substrate of the stream or from surface or subsurface flow through the tailings ponds upstream.

Sulfate (and chloride) and dissolved zinc in the water were above WQSs (MDNR 2000), suggesting that the water quality may have contributed to the impaired community. It appears that the water quality at Eaton Branch was not similar to Cub Creek and was a potential cause for impairment.

4.4 Fine Sediment Estimation

Fine sediment deposition may explain the differences in the metrics between Eaton Branch and Cub Creek. Fine sediments and silt clog the interstitial voids between the larger particles in the substrate and can have destructive effects on invertebrates and fish communities (Chutter 1969; Murphy et al. 1981; Berkman and Rabeni 1987; Smale et al. 1995). According to Zweig and Rabeni (2001), density of observed taxa decreases dramatically with as little as 30 percent fine sediment coverage of the substrate. Fine sediment was observed covering a mean of 78 (±21) percent of the substrate at Eaton Branch, which probably influenced the density. Zweig and Rabeni (2001) also found that taxa richness and EPT taxa were significantly negatively correlated with increased fine sediment on the substrate. Eaton Branch had approximately one-half of the TR, one-third of the EPTT, and one-third of the diversity and evenness of Cub Creek during both seasons. Cub Creek had little fine sediment on the substrate. The high quantity of fine sediment on the substrate at Eaton Branch may have contributed to the impaired macroinvertebrate community.

Interestingly, the BI at Eaton Branch was equal to Cub Creek during both seasons and scored well, which suggested that organic pollution was not responsible for the lower metrics at Eaton Branch. Zweig and Rabeni (2001) found that the BI was not related to fine sediment deposition, which is consistent with this study.

4.5 Fine Sediment Characterization

The fine sediment substrate was characterized for its concentration of heavy metals. Cadmium, lead, and zinc were found in high concentrations in the sediment at Eaton Branch, while sediment metals in Cub Creek were found in low concentrations. Mean concentrations for all three metals exceeded PELs (Ingersoll et al. 1996) at Eaton Branch. Relative concentrations were consistent with sediment sampling and analyses (Cd 76,000; Pb 2,490,000; Zn 3,910,000) conducted by ESP in November 2003 at Eaton Branch (Nodine pers. comm. 2004).

Metals can affect aquatic organisms as toxic substances in water and sediment, or as a toxicant in the food chain (Sorensen 1991; Rainbow 1996). Metals concentrations can be the same or greater in benthic macroinvertebrates as is found in the water (Kiffney and Clements 1993). Kiffney and Clements (1993) suggested that metals sensitivity of macroinvertebrates was related to feeding habits. Herbivorous EPT had the highest metals concentrations, while predators contained lower quantities (Burrows and Whitton 1983; Kiffney and Clements 1993). Besser et al. (1987) stated that aquatic organisms in tributaries of Big River located downstream from tailings piles contained high concentrations of lead, cadmium, and other heavy metals. It appears that the character (metals content) of the fine sediment at Eaton Branch may have been related to the negative metric response.

4.6 Macroinvertebrate Response

Elmid and psephenid beetles were not among the dominant taxa at Eaton Branch, while they were relatively abundant at Cub Creek. Heptageniidae, Isonychidae, and Baetidae mayflies were not prevalent at Eaton Branch, yet were among the dominant taxa at Cub Creek. Zweig and Rabeni (2001) consider these taxa intolerant to excessive fine sediment. The amount of fine sediment on the substrate may have deterred taxa from occupying Eaton Branch.

High levels of dissolved or sediment heavy metals may also have contributed to the impaired community at Eaton Branch. The number of taxa and the abundance of most dominant taxa are reduced and mayflies are significantly reduced (Clements et al. 1988) with exposure to cadmium or zinc. Maret et al. (2003) found that several metals sensitive Ephemeroptera had a significant negative correlation with metals concentrations in water and sediment (r=-0.54 to -0.70) and were significantly lower in number at metals contaminated streams versus reference streams. The most commonly observed changes in benthic communities exposed to metals included reduced Ephemeroptera and concomitant increases in abundance of Chironomindae and/or Hydropyschidae (Clements 1991). When exposed to increasing amounts of heavy metals, the dominant taxon shifts from mayflies to caddisflies to chironomids (Winner et al. 1980). An examination of DMFs in Eaton Branch illustrates very similar results, in that Ephemeroptera were not among the dominant taxa and Chironomidae were found as the highest percentage among the DMFs. Cub Creek contained a variety of mayflies and fewer midges.

The metal sensitivity of mayflies in field and laboratory studies is well established (Winner et al. 1980). Low abundance of Heptagneniidae mayflies is one of the most useful indicators of metals pollution (Clements et al. 2000). Heptageniid mayflies were absent from Eaton Branch and abundant in Cub Creek (Appendix B). *Baetis spp*. also appear to be an extremely sensitive indicator of metal toxicity (Kiffney and Clements 1993). Appendix B shows that *Baetis spp.* were not found in Eaton Branch, however, they were abundant in Cub Creek. Clements et al. (1988) also consider *Isonychia spp*. to be sensitive to heavy metals. *Isonychia spp.* were absent from test stations. Results suggest that either dissolved and/or sediment metals may be impairing the macroinvertebrate community in Eaton Branch.

5.0 Conclusion

In general, the stream habitat at Eaton Branch was not impaired. The Eaton Branch macroinvertebrate community was impaired during both seasons. The water quality may have been impaired by sulfate and chloride and dissolved zinc, which were found above WQSs (MDNR 2000). Fine sediment was observed in large quantities on the substrate and contained high concentrations of heavy metals (cadmium, lead, and zinc) above PELs (Ingersoll et al. 1996). The BI was similar and consistent with the BI at Cub Creek, which suggested that organic pollution was not the contributor to impairment. It appears that Eaton Branch is impaired by constituents from past mining practices and may contribute fine sediment and heavy metals to Big River.

The objectives were met by assessing the macroinvertebrate community integrity, physicochemical water quality, stream habitat, and fine sediment quantity and character. The null hypotheses were addressed. The stream habitat at Eaton Branch was similar to Cub Creek. The macroinvertebrate community integrity at Eaton Branch was not similar to Cub Creek. The physicochemical water quality was not similar. The quantity and character of the fine sediment on the substrate at Eaton Branch was not similar to Cub Creek.

6.0 Recommendations

- Detain fine sediment and water containing heavy metals from entering Eaton Branch.
- Periodically monitor water for heavy metals.
- Conduct biological assessments on other tributaries to Big River that drain mine tailings.

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Appendix A

Missouri Department of Natural Resources Bioassessment and Sediment Study Plan
Eaton Branch
St. Francois County
August 26, 2003

Missouri Department of Natural Resources
Bioassessment and Sediment Study Plan
Eaton Branch, St. Francois County

August 26, 2003

Background

Eaton Branch, St. Francois County, is a small stream that is approximately three miles long in the Ozark/Meramec Ecological Drainage Unit (**EDU**). The stream originates near Wortham, Missouri, and confluences with Big River approximately one mile east of Leadwood, Missouri (Figure 1). Most of the original Eaton Branch, south of Missouri State Highway 8 is now covered with what appears to be mine tailings. It also appears that flow is intermittent or subsurface south of Highway 8. Flow from north of Missouri Highway 8 to the confluence with Big River appears to be permanent or semi-permanent. The stream is a class “C” stream, which may cease flow, but maintain pools in dry weather (MDNR 2000).

Eaton Branch is one tributary of Big River that drains the historic Leadwood tailings ponds in what is known as the Old Lead Belt. The Leadwood mines and mill began operation in 1894 and continued with little interruption until 1965. The tailings ponds consist of approximately 528 acres of inactive lead mine tailings (Fluor Daniel Environmental Services 1995). Two tailings retaining structures capture drainage in the area. Whether or not they are effective is not known. Runoff from these tailing ponds may influence the aquatic communities on Eaton Branch, and subsequently Big River.

While Eaton Branch is not 303 (d) listed, approximately 80 miles of Big River, Washington County is 303 (d) listed for excessive sediment deposition, high lead and zinc values, presumably due to mine tailings runoff. Water runoff during rain events erodes mine wastes, which has increased sedimentation in some lower portions of Big River. Portions of the stream are covered by fine-sediments that virtually eliminates aquatic habitats used by some invertebrates. Fines and silt clog the interstitial voids between the larger particles and can have destructive effects on invertebrates and fish communities (Chutter 1969; Murphy et al. 1981; Berkman and Rabeni 1987; Smale et al. 1995). Metals such as copper, iron, lead, and zinc have been detected in aquatic fauna in areas of Big River. It is not known if the Leadwood tailings ponds upstream on Eaton Branch contribute mine wastes to the stream or to Big River.

It is the Water Quality Monitoring Sections’ intention to determine if mine tailings are impairing aquatic communities in Eaton Branch. Because of the streams small size, a biological comparison will be made to both wadeable/perennial biological criteria and criteria calculated from similar sized control streams. During this study a biological assessment, habitat assessment and fine sediment study will be conducted on Eaton Branch and Cub Creek, a same-size control stream.

Objectives

1) Determine if mine tailings are affecting the macroinvertebrate community and water quality of Eaton Branch.

2) Define the habitat quality of Eaton Branch, St. Francois County.

3) Determine if Eaton Branch is impaired by the quantity or character (metals content) of fine sediment sized particles.

Tasks

1) Conduct a bioassessment, including macroinvertebrates and water physicochemical analyses on Eaton Branch, St. Francois County, and Cub Creek, Washington, County.

2) Conduct a habitat assessment of Eaton Branch and Cub Creek.

3) Conduct a fine sediment percentage estimation and characterization study on Eaton Branch and Cub Creek.

Null Hypotheses

Eaton Branch macroinvertebrate metrics will be similar to biological criteria reference scores, and to the metrics on Cub Creek, a similar size control stream

Water quality at Eaton Branch will be similar to the similar size control stream, Cub Creek #1 and within Water Quality Standards (MDNR 2000).

Habitat assessments at Eaton Branch and Cub Creek will be similar and fully sustainable.

There will be no significant difference ($p > 0.05$) in the fine sediment percentage estimates or character between Eaton Branch and Cub Creek. The metals content will be within acceptable concentrations.

Study Methods

General: The boundary on Eaton Branch, St. Francois County, is between the mouth and 0.5 miles upstream to Missouri State Highway 8 (Figure 1). Two stations will be used in this project for comparisons. The test station on Eaton Branch is located in the SE1/4 of Section 33; T.37N.,R.04E. Cub Creek #1 is the same-size control stream, and is located at the SE1/4 of Section 32; T.36N.,R.01W. Biological assessments, habitat assessments, and a fine sediment study will be conducted on both stations by the Water Quality Monitoring Section (**WQMS**), Environmental Services Program (**ESP**), Air and Land Protection Division (**ALPD**), Missouri Department of Natural Resources (**MDNR**).

Biological Assessment: Macroinvertebrates will be sampled according to the Semi-quantitative Macroinvertebrate Stream Bioassessment Project Procedure (**SMSBPP**; MDNR 2003b). Eaton Branch, St. Francois County is considered a “Riffle/Pool” predominant stream and habitats will be sampled accordingly. Habitats included in these streams are coarse-substrate, non-flow, and rootmat. Each station consists of a length of twenty-times the stream’s average width, which includes at least two riffle sequences. Biological samples will be processed and identified according to MDNR-WQMS-209, Taxonomic Levels for Macroinvertebrate Identifications (MDNR 2001).

Macroinvertebrate data will be compared using two methods. Firstly, Eaton Branch metrics will be a compared with MDNR’s wadeable/perennial stream biological criteria for reference streams in the EDU, and for small control streams. Macroinvertebrate data will be entered in a Microsoft Access database according to the MDNR Standard Operating Procedure WQMS-214, Quality Control Procedures for Data Processing (MDNR 2003a). Data analysis is automated within the Access database, so four standard metrics are calculated: Total Taxa (**TT**); Ephemeroptera, Plecoptera, Trichoptera Taxa (**EPTT**); Biotic Index (**BI**); and the Shannon Diversity Index (**SDI**). Macroinvertebrate data from reference and small control streams within the Ozark/Meramec EDU will allow for the calculation of a 25th percentile for the four metrics in the SMSBPP which are then compared to the Eaton Branch station. Secondly, Stream Condition Indices (**SCI**) for Eaton Branch will be calculated. Each of the four metrics receives a score when compared to the biological criteria. The SCI is derived from the composite score from all four metrics. Eaton Branch will be scored against these calculations and a composite score (SCI) of 16 or greater will determine non-impairment. The SCI at Eaton Branch will be compared to the SCI at Cub Creek, and small control streams in the biological criteria database.

Other comparisons may be conducted to identify impairment. Additional metrics, such as Percent Taxon Similarity, or dominant macroinvertebrate families may be employed to discern differences in taxa between control and test stations.

Biological assessments will be conducted on Eaton Branch and Cub Creek during the fall 2003 and spring 2004 seasons.

Habitat Assessments: A standardized Stream Habitat Assessment Project Procedure (**SHAPP**) will be conducted (MDNR 2003c). Total scores will be compared between streams, and the streams ability to sustain aquatic communities will be identified. Habitat assessments will be conducted at Eaton Branch and Cub Creek once during the study, in the spring of 2003.

Water Quality Sampling: Water will be sampled in Eaton Branch, and Cub Creek. Physicochemical variables determined in the field will include: pH, temperature (C^0), conductivity, dissolved oxygen, and discharge. Water samples collected for analysis include: turbidity, hardness (CaCO3), ammonia-nitrogen, nitrate+nitrite-nitrogen, Total Kjeldahl Nitrogen (**TKN**), chloride, total phosphorus; dissolved barium, calcium,

cadmium, copper, iron, magnesium, lead, and zinc. Samples will be handled according to the Required/Recommended Containers, Volumes, Preservatives, Holding Times, and Special Sampling Considerations (MDNR 2002).

Water samples analyses will be conducted by the Environmental Services Program, Environmental Laboratory in Jefferson City, Missouri. The Water Quality Monitoring Section, will conduct analysis of turbidity. The Chemical and Analytical Section (**CAS**) will conduct the remaining analyses.

Water quality (physicochemical water variables) data will be analyzed using two methods. Water quality data for Eaton Branch will be compared with data from Cub Creek. Results will be compared to Missouri Water Quality Standards (MDNR 2000) and variables not within acceptable limits will be identified.

Water samples will be collected during the fall 2003 and spring 2004 seasons.

Sediment Percentage and Characterization: To ensure sampling method uniformity, depositional areas sampled will be in-stream at the lower margins of riffle/run habitat and upper margins of pools. Depths of the sample areas will not exceed two (2.0) feet and water velocity will be less than 0.5 feet per second (fps). A Marsh-McBirney flow meter will be used to ensure that water velocity of the sample area is within this range. In-stream deposits of fine sediment (i.e. less than particle size ca. 2mm= coarse sand) will be 1) estimated for percent coverage in the substrate and 2) characterized for metals content.

A visual method will be used to estimate the relative percentage of fine sediment per station. Each sampling station will be composed of three sample areas (i.e. grids), each consisting of six contiguous transects across the stream. A tape measure will be stretched from bank to bank at the downstream edge of the grid. One sample quadrat (ca. 10 x 10 inches) will be placed directly on the substrate within each of the six transects using a random number that equates to one foot increments on the tape measure. The trailing edge of the quadrat will be placed on the random foot increment. Two investigators will then estimate the percentage of the stream bottom covered by fine sediment within each quadrat. If the estimated percentages are within ten percent between investigators it will be accepted. If estimates diverge more than ten percent, the investigators will repeat the process until the estimates are within the acceptable margin of error. An average of these two estimates will be recorded and used for analysis.

Sediment will be characterized by determining the total recoverable metals content (TRM- ug/kg) at each of the grids. Specifically, sediments will be analyzed for cadmium, lead and zinc content. Composite collections of sediments will be taken within each grid that are used for fine sediment percentage estimation. If there is not sufficient quantity of fine sediment within the grid (ca. 6 oz.), a representative sample will be collected from an area near the study grid. Each composite will consist of three (3) two-ounce grab samples of sediment. One (1) two-ounce glass jar will be used as a collection device to dredge the bottom to a depth within the sediment of no more than two inches. The sediment sample will be retained for transport through the water column by covering

the opening with the back of the cap. Each sample will be deposited into an eight-ounce glass jar comprising a composite for each transect-grid. There will be three transect-grids per station in order to more accurately characterize and lessen potential bias. Each composite jar will be placed on ice for transport to the ESP Lab according to MDNR-FSS-001 (MDNR 2002).

The percentage of sediment deposition and metals character may be compared between stations, sites, or grids. This will be done by ANOVA on Ranks, non-parametric statistical methods, all pairwise comparison procedures, and possibly correlations, at a significant probability level of $p<0.05$.

The fine sediment percent estimation, and characterization will be conducted one time during the study.

Additional Data Analyses: Ordination of communities with multiple linear regression and correlations may be used in conjunction with habitat assessment, water quality values, sediment percentages, as well as character of sediments in order to correlate with environmental variables.

Data Reporting: A report will be written by the Water Quality Monitoring Section, ESP for the Water Pollution Control Program (**WPCP**).

Quality Controls: All analyses will be done according to recommended Standard Operating Procedures (**SOP**s), Project Procedures (**PP**s), and Quality Assurance Project Procedures (**QAPP**s).

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Attachments: Figure 1: Eaton Branch, St. Francois County, 2003.

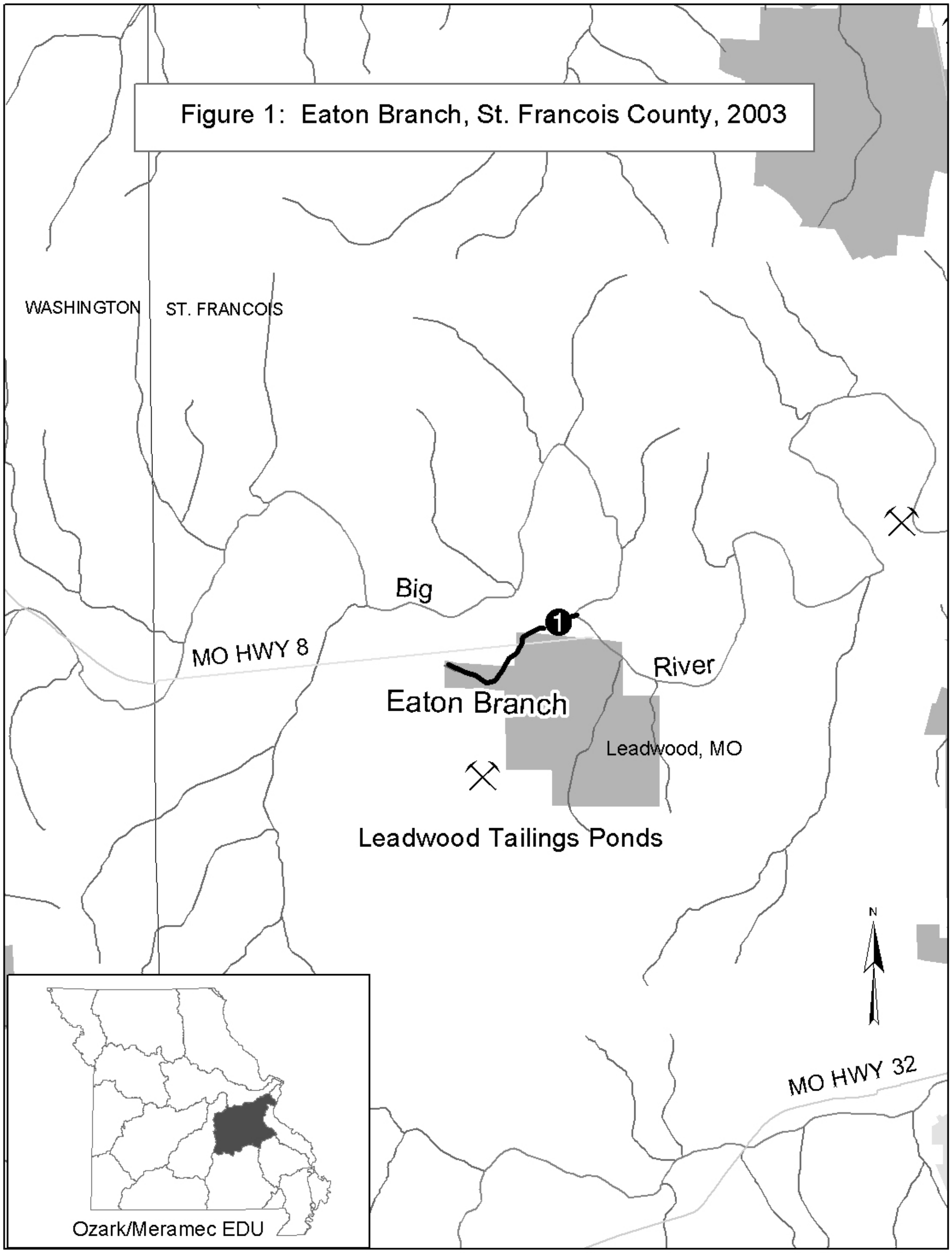
Figure 1: Eaton Branch, St. Francois County, 2003
WASHINGTON
ST. FRANCOIS
Big
River
MO HWY 8
1
Eaton Branch
Leadwood, MO
Leadwood Tailings Ponds
N
MO HWY 32
Ozark/Meramec EDU

Appendix B

Macroinvertebrate Bench Sheets for Eaton Branch and Cub Creek Stations
Fall 2003 and Spring 2004

Aquid Invertebrate Database Bench Sheet Report
Eaton Branch [0318730], Station #1, Sample Date: 10/1/2003 2:10:00 PM

ORDER: TAXA	**CS**	**NF**	**SG**	**RM**
AMPHIPODA				
Stygobromus				1
COLEOPTERA				
Berosus	4			
Hydroporus	1	6		1
DIPTERA				
Ceratopogoninae				1
Chaetocladius	1			
Clinotanypus				1
Cricotopus/Orthocladius	1			
Dicrotendipes	1			
Empididae	1			
Erioptera	7			
Hydrobaenus		5		8
Larsia	1			2
Parametriocnemus	11	1		1
Phaenopsectra	2	2		1
Polypedilum convictum grp	1			
Psectrocladius	282	51		17
Simulium	2			
Thienemannimyia grp.	1			
Tipula	12			
Zavrelimyia				1
ISOPODA				
Caecidotea	1			20
MEGALOPTERA				
Sialis				1
ODONATA				
Argia	16	3		40
Boyeria				1
Enallagma	1			3
Gomphus	2			1
Hetaerina	5			3
Libellula		1		
Macromia				3
TRICHOPTERA				
Ceratopsyche	4			
Cheumatopsyche	3			
Oecetis	13	1		21
Oxyethira	8			3
Phryganeidae				5
Polycentropus	1			

ORDER: TAXA	CS	NF	SG	RM
Triaenodes	1			73
TUBIFICIDA				
Branchiura sowerbyi		1		
Limnodrilus hoffmeisteri		2		
Tubificidae	2	4		59

Aquid Invertebrate Database Bench Sheet Report
Cub Ck [0318731], Station #1, Sample Date: 10/2/2003 12:30:00 PM

ORDER: TAXA	**CS**	**NF**	**SG**	**RM**
N/A				
Branchiobdellida				3
"HYDRACARINA"				
Acarina	5			3
AMPHIPODA				
Hyalella azteca				30
Stygobromus		1		
COLEOPTERA				
Ancyronyx variegatus				4
Dubiraphia		13		35
Helichus basalis				3
Helichus lithophilus				1
Macronychus glabratus				9
Optioservus sandersoni	129	4		5
Psephenus herricki	245	56		1
Scirtes				16
Stenelmis	47	2		8
DECAPODA				
Orconectes luteus		1		1
Orconectes medius	3	-99		
Orconectes punctimanus		1		
DIPTERA				
Anopheles				1
Atherix	-99			
Ceratopogoninae		4		2
Chironomus		1		
Corynoneura		1		7
Cricotopus/Orthocladius	21	1		5
Dicrotendipes		1		2
Dixella				8
Hemerodromia				2
Labrundinia				5
Microtendipes		13		2
Myxosargus				2
Parakiefferiella		1		
Parametriocnemus		12		1
Paratanytarsus				4
Pilaria				1
Polypedilum convictum grp	2			
Polypedilum halterale grp		2		
Polypedilum illinoense grp				3
Potthastia	2			

ORDER: TAXA	CS	NF	SG	RM
Procladius		1		
Pseudochironomus		1		
Rheotanytarsus	4			1
Simulium	15			2
Stempellinella	1			
Stenochironomus		2		5
Tabanus		1		
Tanytarsus		5		7
Thienemanniella	1	1		
Thienemannimyia grp.	1	11		8
Tipula	-99			
Tribelos		23		6
Zavrelimyia				2
EPHEMEROPTERA				
Acentrella	10			
Baetidae				1
Baetis	37			
Caenis anceps	3	8		2
Caenis latipennis		6		7
Ephemerellidae	1			
Eurylophella				6
Heptageniidae	7			4
Isonychia bicolor	46			1
Leptophlebiidae		4		10
Stenacron	2	36		1
Stenonema bednariki	7			
Stenonema femoratum		11		1
Stenonema mediopunctatum	22			
Stenonema pulchellum	8			
Tricorythodes	2			
HEMIPTERA				
Metrobates		2		
Microvelia				4
ISOPODA				
Caecidotea				3
LIMNOPHILA				
Ancylidae		2		4
Fossaria				3
Menetus		1		2
Physella				2
Planorbidae		1		
LUMBRICINA				
Lumbricidae	-99	6		
LUMBRICULIDA				

ORDER: TAXA	CS	NF	SG	RM
Lumbriculidae				1
MEGALOPTERA				
Corydalus	1			
MESOGASTROPODA				
Elimia	9			-99
ODONATA				
Argia		9		
Boyeria				2
Gomphidae	2			
Hagenius brevistylus		3		
Hetaerina		2		7
Macromia				1
PLECOPTERA				
Pteronarcys pictetii	-99			
TRICHOPTERA				
Cheumatopsyche	8			
Chimarra	1	5		
Helicopsyche	8			6
Limnephilidae				10
Lype diversa				2
Nectopsyche				1
Oecetis				3
Polycentropus	2	4		2
Psychomyia	1			
Triaenodes				9
TRICLADIDA				
Planariidae	10			1
TUBIFICIDA				
Tubificidae		1		7
VENEROIDEA				
Pisidium		5		
Sphaerium				5

Aquid Invertebrate Database Bench Sheet Report
Eaton Branch [0418659], Station #1, Sample Date: 3/23/2004 11:45:00 AM

ORDER: TAXA	**CS**	**NF**	**SG**	**RM**
AMPHIPODA				
Crangonyx	13			
COLEOPTERA				
Dytiscus	1			
Haliplus		1		
Hydroporus		2		3
DIPTERA				
Ablabesmyia				2
Ceratopogoninae	6	7		3
Chaetocladius	4	3		1
Clinocera	13	1		
Corynoneura				1
Cricotopus bicinctus	1			
Cricotopus/Orthocladius	42	1		5
Cryptotendipes		1		
Diamesa	7			
Dicrotendipes		2		
Diptera	1			
Hemerodromia	1			
Parakiefferiella	1			
Parametriocnemus	34	1		
Paraphaenocladius	1	2		
Polypedilum illinoense grp				1
Prosimulium	4			
Psectrocladius	348	253		226
Simulium	30			
Smittia		1		
Thienemanniella	1			
Thienemannimyia grp.		1		
Tipula	1	-99		-99
EPHEMEROPTERA				
Caenis latipennis	1	2		12
Callibaetis				1
Leptophlebia		1		
HEMIPTERA				
Microvelia				1
ISOPODA				
Caecidotea	5			
LIMNOPHILA				
Physella		1		
ODONATA				
Argia				16

ORDER: TAXA	CS	NF	SG	RM
Enallagma				7
Gomphus				1
Libellula		1		
PLECOPTERA				
Amphinemura	64			
Perlesta	13			
TRICHOPTERA				
Hydropsyche	1			
Oecetis	1			5
Oxyethira	4			10
Triaenodes				6
TRICLADIDA				
Planariidae				1
TUBIFICIDA				
Branchiura sowerbyi	1	3		
Enchytraeidae	1	1		
Limnodrilus hoffmeisteri	10	11		
Tubificidae	19	20		1

Aquid Invertebrate Database Bench Sheet Report
Cub Ck [0418660], Station #1a, Sample Date: 3/23/2004 4:30:00 PM

ORDER: TAXA	**CS**	**NF**	**SG**	**RM**
"HYDRACARINA"				
Acarina	15	2		6
AMPHIPODA				
Allocrangonyx		1		
Hyalella azteca				2
Stygobromus		2		
COLEOPTERA				
Ancyronyx variegatus				1
Dubiraphia		5		7
Macronychus glabratus				2
Optioservus sandersoni	129	20		5
Paracymus		1		
Psephenus herricki	3	2		1
Stenelmis	4			
DECAPODA				
Orconectes medius	-99			
Orconectes punctimanus				1
DIPTERA				
Ablabesmyia		1		
Atherix	-99			
Ceratopogoninae		12		3
Clinocera		5		6
Corynoneura	4	3		18
Cricotopus bicinctus				4
Cricotopus/Orthocladius	51	11		35
Cryptochironomus		1		
Dixella				2
Eukiefferiella	51	38		2
Hemerodromia	11	1		1
Labrundinia				3
Micropsectra		1		
Microtendipes				1
Natarsia		2		
Nilotanypus				1
Orthocladius (Euorthocladius)	2			
Parametriocnemus	2	1		
Paratanytarsus				1
Paratendipes		1		
Phaenopsectra		2		
Polypedilum convictum grp	11			1
Polypedilum halterale grp		2		
Polypedilum illinoense grp				1

ORDER: TAXA	CS	NF	SG	RM
Polypedilum scalaenum grp		1		
Potthastia	19	49		19
Prosimulium	2			
Rheocricotopus	14			5
Rheotanytarsus	2			9
Simulium	2			1
Stempellinella	2	8		1
Sympotthastia	11	5		8
Tabanus		-99		
Tanytarsus		9		
Thienemanniella	3			12
Thienemannimyia grp.	5	23		11
Tvetenia	1			
Zavrelimyia		1		
EPHEMEROPTERA				
Acentrella	14			1
Caenis latipennis		3		2
Centroptilum		1		1
Ephemerella invaria	12			1
Eurylophella bicolor	4	3		11
Heptageniidae	4			
Isonychia bicolor	4			1
Leptophlebia		1		1
Stenacron	1			
Stenonema bednariki	2			
Stenonema femoratum				2
Stenonema mediopunctatum	3			1
Stenonema pulchellum	4			6
ISOPODA				
Caecidotea				1
LIMNOPHILA				
Ancylidae		1		
Fossaria		1		
Physella				-99
LUMBRICINA				
Lumbricidae	1	5		
LUMBRICULIDA				
Lumbriculidae		5		
MEGALOPTERA				
Corydalus	-99			
MESOGASTROPODA				
Elimia	2	1		9
ODONATA				

ORDER: TAXA	CS	NF	SG	RM
Argia	1			
Basiaeschna janata				-99
Calopteryx				1
Gomphidae	1			
PLECOPTERA				
Acroneuria				-99
Amphinemura	69			31
Leuctra	134	28		3
Perlidae	1			16
Perlinella ephyre		1		
Prostoia	2			
Pteronarcys pictetii	8			2
Strophopteryx	1			
TRICHOPTERA				
Cheumatopsyche	9			
Chimarra	2			
Helicopsyche	2			4
Hydroptila	5			6
Polycentropus		1		4
Psychomyia				2
Ptilostomis				-99
Pycnopsyche				3
Rhyacophila	2			
TRICLADIDA				
Planariidae	6			1
TUBIFICIDA				
Limnodrilus hoffmeisteri				2
Tubificidae		1		6
VENEROIDEA				
Sphaeriidae		1		3

Aquid Invertebrate Database Bench Sheet Report
Cub Ck [0418661], Station #1b, Sample Date: 3/23/2004 4:30:00 PM

ORDER: TAXA	**CS**	**NF**	**SG**	**RM**
"HYDRACARINA"				
Acarina	6	2		5
AMPHIPODA				
Hyalella azteca				1
COLEOPTERA				
Dubiraphia		3		3
Hydroporus		1		
Macronychus glabratus				1
Optioservus sandersoni	91	17		5
Psephenus herricki	3	1		2
Stenelmis	1			
DECAPODA				
Orconectes luteus		-99		-99
Orconectes medius	-99			
DIPTERA				
Ablabesmyia		3		2
Atherix	-99			
Ceratopogoninae		9		1
Chelifera				1
Clinocera	5	7		5
Corynoneura	1	7		25
Cricotopus/Orthocladius	43	21		31
Cryptochironomus		3		
Dixella				1
Eukiefferiella	18			
Hemerodromia		1		
Labrundinia				6
Natarsia		1		
Nilotanypus	1			
Ormosia				1
Orthocladius (Euorthocladius)	1			1
Parametriocnemus	2			
Paratanytarsus		1		1
Paratendipes		1		
Polypedilum convictum grp	6			1
Polypedilum halterale grp		1		
Potthastia	3	83		19
Prosimulium	1			
Psectrocladius				1
Rheocricotopus	9			1
Rheotanytarsus	3			7
Simulium	4			

ORDER: TAXA	CS	NF	SG	RM
Stempellinella	1	10		7
Sympotthastia	8	5		6
Synorthocladius				1
Tabanus	-99	2		
Tanytarsus	1	14		1
Thienemanniella	1			14
Thienemannimyia grp.	3	21		22
Tribelos		8		
Tvetenia	3			
Zavrelimyia				1
EPHEMEROPTERA				
Acentrella	15			1
Caenis latipennis				2
Centroptilum				2
Ephemerella invaria	5			2
Eurylophella bicolor		4		7
Heptageniidae	1			
Isonychia bicolor	3			
Leptophlebia		2		7
Paraleptophlebia				3
Stenacron		2		1
Stenonema bednariki	1			
Stenonema femoratum		2		7
Stenonema mediopunctatum	1			
Stenonema pulchellum	5	1		6
ISOPODA				
Caecidotea				1
LIMNOPHILA				
Physella				-99
LUMBRICINA				
Lumbricidae	1	9		1
MEGALOPTERA				
Corydalus	-99			
Nigronia serricornis				1
MESOGASTROPODA				
Elimia	-99			16
ODONATA				
Boyeria				4
Calopteryx				1
Stylogomphus albistylus		2		1
PLECOPTERA				
Amphinemura	20			9
Leuctra	39	22		2

ORDER: TAXA	CS	NF	SG	RM
Perlidae				5
Prostoia	1			1
Pteronarcys pictetii	1			1
TRICHOPTERA				
Cheumatopsyche	9	1		1
Chimarra	5			
Helicopsyche	6			4
Hydroptila				6
Lype diversa				1
Oecetis	1			
Polycentropus	1	2		
Pycnopsyche		1		6
TRICLADIDA				
Planariidae		1		1
TUBIFICIDA				
Enchytraeidae				1
Limnodrilus hoffmeisteri		2		
Tubificidae		16		1
VENEROIDEA				
Pisidium		1		1

Appendix C

Fine Sediment Percentage and Sediment Character (metals) Tests:
Mann-Whitney Rank and Student t-tests for Eaton Branch and Cub Creek
October 1 and 2, 2003

t-test Wednesday, March 03, 2004, 09:59:14

Data source: Percent fine sediment per stream

Normality Test: Failed (P = <0.001)

Test execution ended by user request, Rank Sum Test begun

Mann-Whitney Rank Sum Test Wednesday, March 03, 2004, 09:59:14

Data source: Percent fine sediment per stream

Group	N	Missing	Median	25%	75%
Eaton	18	0	87.000	68.000	96.000
Cub	18	0	3.000	2.000	6.000

T = 495.000 n(small)= 18 n(big)= 18 (P = <0.001)

The difference in the median values between the two groups is greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

t-test Wednesday, March 03, 2004, 09:45:55

Data source: Cadmium (sediment) per stream

Normality Test: Passed (P = 0.036)

Equal Variance Test: Passed (P = 0.099)

Group Name	N	Missing	Mean	Std Dev	SEM
Eaton	3	0	67100.000	10801.852	6236.452
Cub	3	0	60.400	5.303	3.062

Difference 67039.600

t = 10.750 with 4 degrees of freedom. (P = <0.001)

95 percent confidence interval for difference of means: 49724.432 to 84354.768

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

t-test Wednesday, March 03, 2004, 09:50:14

Data source: Lead (sediment) per stream

Normality Test: Passed (P = 0.040)

Equal Variance Test: Passed (P = 0.122)

Group Name	N	Missing	Mean	Std Dev	SEM
Eaton	3	0	2243333.333	993243.844	573449.601
Cub	3	0	12843.333	9843.091	5682.911

Difference 2230490.000

t = 3.889 with 4 degrees of freedom. (P = 0.018)

95 percent confidence interval for difference of means: 638260.506 to 3822719.494

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.018).

Power of performed test with alpha = 0.050: 0.805

t-test Wednesday, March 03, 2004, 09:52:23

Data source: Zinc (sediment) per stream

Normality Test: Passed (P = 0.037)

Equal Variance Test: Passed (P = 0.343)

Group Name	N	Missing	Mean	Std Dev	SEM
Eaton	3	0	2013333.333	579511.288	334580.998
Cub	3	0	16833.333	929.157	536.449

Difference 1996500.000

t = 5.967 with 4 degrees of freedom. (P = 0.004)

95 percent confidence interval for difference of means: 1067553.044 to 2925446.956

The difference in the mean values of the two groups is greater than would be expected by chance; there is a statistically significant difference between the input groups (P = 0.004).

Power of performed test with alpha = 0.050: 0.990